or modification. MPEP 2143.01 (emphasis added) (citing *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990)).

Claim 1, the only independent claim in the present application, reads as follows:

> 1. A method of transfecting dendritic cells comprising:
> providing dendritic cells;
> providing a transfection agent comprising a polynucleotide and microparticles, said microparticles comprising biodegradable polymer and cationic detergent; and
> incubating the dendritic cells and transfection agent *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide.

Song et al., the primary reference relied upon in the rejection, proposes several gene delivery vehicles including: (a) gene delivery by viral methods, and (b) gene delivery where the expression vector is not carried by a virus, such as (i) a eukaryotic layered vector initiation system, (ii) complexation of the expression vector with one or more polynucleotide condensing agents such as polycations, and (iii) association of the expression vector with lipids, preferably encapsulation within liposomes, particularly liposomes made of cationic lipids. See Song et al. at page 2, lines 16 *et seq.*

However, according to Song et al., direct injection of recombinant retroviruses is preferred. See, e.g., Song et al., page 27, lines 25-27. Moreover, among the numerous non-viral techniques taught by Song et al, none appears to use a transfection agent comprising a polynucleotide and microparticles as claimed.

The Office Action notes that Song et al. does not disclose the use of a transfection agent comprising a polynucleotide in combination with microparticles that further comprise a biodegradable polymer and a cationic detergent, and turns to Hedley et al. and Fattal et al. in an attempt to make up for this deficiency in Song et al.

Hedley et al. teaches a number of microparticle administration schemes. However, there is no motivation in Hedley et al. to introduce microparticles into dendritic cells. Indeed, it appears that dendritic cells are only mentioned once in all of Hedley et al, and then only in connection with numerous administration schemes that are not said to relate to dendritic cells. Moreover, it is noted that the techniques are *in vivo* rather than *ex vivo* as claimed. Accordingly, upon reviewing Hedley et al., one of ordinary skill in

the art would not be motivated to transfect dendritic cells with microparticles as presently claimed, nor would that person perform the transfection in an *ex vivo* fashion.

Furthermore, the teachings of Hedley et al. do not provide a reasonable expectation that dendritic cells can be successfully transfected with microparticles as presently claimed. For instance, Example 2 of Hedley et al. indicates that DNA is phagocytosed and expressed by *macrophages*. However, this would not create a reasonable expectation of success vis-à-vis *dendritic cells*, because dendritic cells are notoriously resistant to transfection using *ex vivo* nonviral techniques. See, e.g., the present specification at page 6, lines 6-10. See also the Abstract [copy attached] of Denis-Mize K.S., et al., *Gene Ther* 2000 Dec;7(24):2105-12, which notes that "DC [dendritic cells] are not readily transfected in vitro by traditional nonviral techniques."

In addition, and as recognized in the Office Action, Hedley et al. does not teach or suggest the use of microparticles that contain cationic detergent as claimed. The Office turns to Fattal et al. to make up for this deficiency in Hedley et al. In particular, the Office Action maintains that one of ordinary skill in the art upon reading Fattal et al. would include a cationic detergent such as CTAB in the microparticles of Hedley et al. in order to (a) increase the amount polynucleotide associated with the polymer particles and (b) increase the uptake of the microparticles by phagocytosis. Applicants respectfully disagree.

As to increasing the uptake of the microparticles by phagocytosis, Applicants note that the cited portion of Fattal et al. (page 137) suggests that uptake of antisense oligonucleotide is increased due its association with a nanoparticle, rather than suggesting that the cationic detergent acts to increase the uptake of the nanoparticle by phagocytosis. See also the first full paragraph of p. 140 of Fattal et al.

Regarding increasing the amount polynucleotide, it is noted that Fattal et al. *adsorbs* antisense oligonucleotide on polymer particles. In contrast, Hedley et al., which the Office proposes to combine with Fattal et al., does not *adsorb* DNA onto the microparticles disclosed therein, but rather *encapsulates* the DNA within the

microparticles.[1] Hence, contrary to the Office Action, one of ordinary skill in the art would not be motivated to modify the microparticles of Hedley et al. by the addition of the cationic detergent from Fattal et al. to increase the association of the DNA with the microparticles.

Furthermore, the asserted success of Fattal et al. in transfecting U937 cells (commonly referred to in the art as "monocyte-like" or "macrophage-like" cells) with antisense oligonucleotides would not lead to the expectation that dendritic cells can be successfully transfected with polynucleotides, because, as noted above in connection with Hedley et al., dendritic cells are notoriously difficult to transfect using non-viral methods.

Hence, a *prima facie* case of obviousness has not been established with respect to the presently pending claims because (a) there is not motivation to combine the various features of Song et al., Hedley et al. and Fattal et al. to arrive at the present invention, and (b) there is no reasonable expectation of success in such an endeavor.

Reconsideration of the outstanding rejection of claims 1-23 and 29-31 under 35 U.S.C. 103(a) are therefore respectfully requested.

CONCLUSION

Claims 1-53 are in condition for allowance, notification of which is earnestly solicited. The Examiner is invited to telephone the Applicant's attorney at (703) 433-0510 to resolve any outstanding issues in this case.

FEES

The Office is authorized to charge any required fees, including the $400.00 fee for a two-month extension of time, to deposit account number 50-1047.

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[1] See, e.g., col. 9, lines 2-4 ("microparticles can be prepared which carry ... DNA ... *within* each microparticle"), col. 13, lines 64-66 ("the protein or peptide encoded by the nucleic acid contained *within* the microparticle"), Table 5 ("Phagocytosis of *encapsulated* DNA leads to expression of a luciferase reporter gene construct") and Table 6 ("Expression of *encapsulated* luciferase DNA in murine muscles") (emphasis added).

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Serial No. 09/715,902
Docket No. PP01627.003

Version with Markings to Show Changes Made

1. (Amended) A method of transfecting dendritic cells comprising:

providing dendritic cells;

providing a transfection agent comprising a polynucleotide and microparticles, said microparticles ~~comprised of~~ comprising biodegradable polymer and cationic detergent; and

incubating the dendritic cells and transfection agent *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide.

2. The method of claim 1, wherein the dendritic cells originate from bone marrow.

3. The method of claim 1, wherein the dendritic cells originate from blood.

4. The method of claim 1, wherein the dendritic cells originate from a vertebrate subject.

5. (Amended) The method of claim 1, wherein the dendritic cells ~~are~~ originate from a human subject.

6. (Amended) The method of claim 1, wherein the cationic detergent ~~comprises~~ ~~CTAB~~ is cetyl trimethyl ammonium bromide.

7. (Amended) The method of claim 1, wherein the cationic detergent ~~comprises~~ is cetrimide.

8. The method of claim 1, wherein the polymer is a poly(α-hydroxy acid).

9. The method of claim 1, wherein the polymer is a poly(lactide).

10. The method of claim 1, wherein the polymer is a copolymer of D,L-lactide and glycolide or glycolic acid.

11. The method of claim 1, wherein the polymer is a poly(D,L-lactide-co-glycolide).

12. The method of claim 1, wherein the polymer is a copolymer of D,L-lactide and caprolactone.

13. The method of claim 1, wherein the dendritic cells are cultured for about 5 days prior to transfection.

14. (Amended) The method of claim 1, wherein the dendritic cells are cultured for about 5 to about 10 days prior to transfection.

15. The method of claim 1, wherein the dendritic cells and transfecting agent are incubated for about 24 hours.

16. (Amended) The method of claim 1, wherein said polynucleotide is provided in the form of a plasmid.

17. (Amended) The method of claim 1, wherein said polynucleotide encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor.

18. (Amended) The method of claim 17, wherein the antigen is associated with ~~HIV~~ human immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, human papillomavirus, influenza A virus, meningitis A, meningitis B, or meningitis C.

19. A method for producing an immune response comprising administering, to a vertebrate subject in need thereof, an effective amount of dendritic cells produced by the method of claim 17.

20. The method according to claim 19, in which the dendritic cells originate from the vertebrate subject.

21. The method according to claim 19, in which the dendritic cells originate from a healthy vertebrate subject MHC-matched to the vertebrate subject.

22. The method according to claim 19, in which the dendritic cells are administered parenterally.

23. The method according to claim 19, in which the dendritic cells are administered by direct injection into affected tissue.

24. A method for producing an immune response in a vertebrate subject in need thereof comprising:

providing T cells;

activating said T cells by subjecting them to the dendritic cells produced by the method of claim 17;

administering said activated T cells to said subject.

25. The method according to claim 24, in which the dendritic cells and T cells originate from the vertebrate subject.

26. The method according to claim 24, in which the dendritic cells and T cells originate from a healthy vertebrate subject MHC-matched to the vertebrate subject.

27. The method according to claim 24, in which the T cells are administered parenterally.

28. The method according to claim 24, in which the T cells are administered by direct injection into affected tissue.

29. Antigen presenting dendritic cells made by the method of claim 17.

30. (Amended) The method according to claim 1, wherein said microparticles have ~~transfection agent has a~~ diameters ranging from about 500 nm to about 30 μm. ~~on the order of 1 micron.~~

31. The method according to claim 1, wherein said transfection agent contains on the order of 1% w/w polynucleotide.

32. (Newly added) The method of claim 17, wherein the detergent is cetyl trimethyl ammonium bromide.

33. (Newly added) The method of claim 17, wherein said polynucleotide encodes a viral antigen.

34. (Newly added) The method of claim 17, wherein said polynucleotide encodes a tumor antigen.

35. (Newly added) The method of claim 17, wherein said polynucleotide encodes a bacterial antigen.

36. (Newly added) The method of claim 17, wherein said polynucleotide encodes a parasitic antigen.

37. (Newly added) The method of claim 17, wherein said polynucleotide encodes a fungal antigen.

38. (Newly added) The method of claim 19, wherein said polynucleotide encodes a viral antigen.

39. (Newly added) The method of claim 19, wherein said polynucleotide encodes a tumor antigen.

40. (Newly added) The method of claim 19, wherein said polynucleotide encodes a bacterial antigen.

41. (Newly added) The method of claim 19, wherein said polynucleotide encodes a parasitic antigen.

42. (Newly added) The method of claim 19, wherein said polynucleotide encodes a fungal antigen.

43. (Newly added) The method of claim 19, wherein said polynucleotide encodes a human immunodeficiency virus antigen, a herpes simplex virus antigen, a hepatitis B virus antigen, a hepatitis C virus antigen, a human papillomavirus antigen, an influenza A virus antigen, a meningitis A antigen, a meningitis B antigen, or a meningitis C antigen.

44. (Newly added) The method of claim 19, wherein the detergent is cetyl trimethyl ammonium bromide.

45. (Newly added) The method of any of claims 1-23 and 29-44, wherein at least a portion of said polynucleotide is adsorbed on the surface of said microparticles.

46. (Newly added) The method of claim 1, wherein at least a portion of said polynucleotide is entrapped within said microparticles.

47. (Newly added) The method of claim 1, wherein at least a portion of said polynucleotide is both adsorbed on the surface of said microparticles and entrapped within said microparticles.

48. (Newly added) The method of claim 17, wherein at least a portion of said polynucleotide is entrapped within said microparticles.

49. (Newly added) The method of claim 17, wherein at least a portion of said polynucleotide is both adsorbed on the surface of said microparticles and entrapped within said microparticles.

50. (Newly added) The method of claim 19, wherein at least a portion of said polynucleotide is entrapped within said microparticles.

51. (Newly added) The method of claim 19, wherein at least a portion of said polynucleotide is both adsorbed on the surface of said microparticles and entrapped within said microparticles.

52. (Newly added) The method of any of claims 1-7, 13-23, 29-44 and 46-51, wherein the polymer is a poly(lactide-co-glycolide).

53. (Newly added) The method of any of claims 1-15, 19-23, 29-32, 44 and 46-51 wherein the polynucleotide is an expression vector encoding an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor.

Gene Ther 2000 Dec;7(24):2105-12 Related Articles, Links

nature publishing group

Plasmid DNA adsorbed onto cationic microparticles mediates target gene expression and antigen presentation by dendritic cells.

Denis-Mize KS, Dupuis M, MacKichan ML, Singh M, Doe B, O'Hagan D, Ulmer JB, Donnelly JJ, McDonald DM, Ott G.

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Dendritic cells (DC) play a key role in antigen presentation and activation of specific immunity. Much current research focuses on harnessing the potency of DC for vaccines, gene therapy, and cancer immunotherapy applications. However, DC are not readily transfected in vitro by traditional nonviral techniques. A novel DNA vaccine formulation was used to determine if DC are transfected in vitro. The formulation consists of plasmid DNA adsorbed on to cationic microparticles composed of the biodegradable polymer polylactide-co-glycolide (PLG) and the cationic surfactant, cetyltrimethylammonium bromide (CTAB). Using preparations of fluorescent-labeled plasmid DNA formulated on PLG-CTAB microparticles to study internalization by macrophages and dendritic cells in vitro and in vivo, we found that most, but not all, of the fluorescence was concentrated in endosomal compartments. Furthermore, uptake of plasmid DNA encoding HIV p55 gag adsorbed to PLG-CTAB microparticles by murine bone marrow-derived dendritic cells resulted in target gene expression, as detected by RT-PCR. The antigen was subsequently processed and presented, resulting in stimulation of an H-2kd-restricted, gag-specific T cell hybridoma. Activation of the hybridoma, detected by IL-2 production, was dose-dependent in the range of 0.1-20 microg DNA (10-2000 microg PLG) and was sustained up to 5 days after transfection. Thus, adsorption of plasmid DNA on PLG-CTAB microparticles provides a potentially useful nonviral approach for in vitro transfection of dendritic cells. Gene Therapy (2000) 7, 2105-2112.

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